3D Collagen I Matrix Kit
(3D-CMK)
Cat #8688

Introduction

Collagen is a major structural component in the extracellular matrix (ECM) and in connective tissues such as tendons, ligaments, dermis, and blood vessels. As insoluble fibrous proteins, collagens are the primary determinant of ECM tensile strength and they help tissues withstand stretching. While there are at least 28 types of collagen, 80-90% of the collagen in the human body are types I, II, and III. The 3D Collagen I Matrix Kit (3D-CMK) contains collagen type I, which is found in skin, tendon, vasculature, and bone ECM, as well as the accessory components needed to form a 3D collagen gel matrix. Note: for inclusive kits to create 3-dimensional scaffolds with primary cells, see ScienCell™'s 3D Embedded Tubule Formation Kit (Cat. #8708), ScienCell™'s Network Formation Assay Kit (Cat. #8718), and ScienCell™'s Endothelial–Pericyte Coculturing Kit (Cat. #8728).

Kit Components

The ScienCell™ 3D Collagen I Matrix Kit contains collagen I purified from rat tail tendon, 10X Buffer A, Buffer B, and sterile H_2O.

Cat. #	# of vials	Name	Quantity	Storage
8688-a	1	Collagen I from rat tail, 4mg/ml	25 ml	2-8°C
8688-b	1	Buffer A, 10x	5 ml	2-8°C
8688-c	1	Buffer B	1 ml	2-8°C
8688-d	3	Sterile H_2O	5 ml	2-8°C

Note: See ScienCell™ Cat #8708 (3D-ETF), #8718 (3D-NF), and #8728 (3D-EPC) for complete and inclusive 3D collagen-based kits to assess endothelial network formation and tubule formation.

Quality Control

3D-CMK is tested for gel formation and HUVEC tubule formation. It is negative for bacterial and fungal contamination.

Product Use

3D-CMK is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Shipping

3D-CMK is shipped on gel ice.

Procedure: Preparation time ~1 hr, designed for 24-well plates

Important notes before starting procedure

- Keep all kit components chilled on ice until ready for use
- We recommend making about 500 µL of extra gel to account for gel lost during pipetting
- Gel polymerization is affected by temperature
- All work is to be done in a sterile flow hood to maintain sterility; surfaces should be decontaminated prior to entry and no components should be opened outside of the sterile area
- If possible, keep all liquid components on ice throughout procedure until ready for plating
    - Gelling occurs rapidly at room temperature
    - If possible, perform all component manipulations on ice in a sterile flow hood
- Protocol is designed to make 1 mL of 2 mg/mL collagen gel
    - Designed for gels in 24-well plates
    - Each well requires 300 µL of gel
        - Gel thickness may be adjusted by adding more or less gel per well
    - Please scale appropriately
    - Calculations may be done to adjust collagen concentration in gels
        - Refer to note at end of protocol
- Cells and cell culturing media are *not* included in this kit

A. Preparation of cells

1. Count and centrifuge cells to pellet
    a. The optimal number of cells may depend on the cell type and application, and should be titrated to determine the optimal density.
    b. Leave the excess media with the cell pellet and remove just prior to collagen addition before plating.

B. Preparation of gel

1. Prepare gel components by combining 500 µL collagen (Cat. #8688-a), 100 µL Buffer A (Cat. #8688-b), and 390 µL sterile H_2O (8688-d)
    1.1. Mix contents well with gentle pipetting; avoid bubbles
2. Prepare an uncoated, sterile 24-well plate(s) for plating
3. Retrieve pelleted cells from Step A and remove excess medium.

********AFTER THIS NEXT STEP, action needs to BE AS QUICK AS POSSIBLE without sacrificing care********

4. To the mixture from Step 1, add 10 µL Buffer B (8688-c)
    4.1. Mix well with gentle pipetting; avoid bubbles
    4.2. BE QUICK; gel starts to polymerize immediately with addition of Buffer B
5. Add the appropriate amount of gel to cells to obtain the desired cell density.
    5.1. Mix well by gentle pipetting; avoid bubbles.
6. Pipette 300 µL of gel mixture from step 3 to the middle of each well of a 24-well plate
7. Carefully place the plate in a 37 °C/5% CO_2 humidity incubator and let the gel polymerize for 1 hour
8. Submerge polymerized gel with appropriate cell culturing medium
    8.1. Aggressive addition of medium can dislodge the gel
    8.2. Cold media can disrupt the integrity of the gel

8.3. Use enough culturing medium to cover the entire gel

9. Maintain gels in a 37 °C/5% CO_2 humidity incubator and change the medium every other day
   9.1. Do not use a vacuum aspirator as aggressive aspiration can dislodge the gel
   9.2. Remove medium using a pipette by hand

Note: To adjust collagen concentration in gels, determine the total volume of collagen needed and then determine the amount of reagents necessary to achieve a normal osmolality and pH.

To determine the volume of components needed:

- Volume of collagen (Vc) = $\frac{\text{(desired final collagen concentration) x [total desired volume (Vt)]}}{\text{initial concentration of collagen (4 mg/mL)}}$
- Volume of Buffer A (Va) = $\frac{\text{total desired volume (Vt)}}{10}$
- Volume of Buffer B (Vb) = (Vc) x 0.023
- Volume of sterile H_2O (Vh) = (Vt) – (Vc) – (Va) – (Vb)